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<b>(54) Title:</b> METHODS AND COMPOSITIONS EMPLOYING RED YEAST FERMENTATION PRODUCTS  <b>(57) Abstract</b>  Methods and compositions are disclosed which comprise red yeast fermentation products, that can be used as natural dietary supplements and/or medicaments for the treatment or prevention of hyperlipidemia and associated disorders and symptoms, such as cardiovascular diseases, cerebrovascular diseases, diabetes, hypertension, obesity, asthenic breathing, chronic headache, chest pain and tightness, limb swelling and distention, loss of appetite and excess expectoration. The methods and compositions are effective in lowering both the serum cholesterol and serum triglyceride levels in humans, and can be used for maintaining cardiovascular health. The invention also encompasses particular <i>Monascus</i> strains that yield fermentation products with the desired biological activities.		

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**METHODS AND COMPOSITIONS EMPLOYING  
RED YEAST FERMENTATION PRODUCTS**

**1. TECHNICAL FIELD**

5       The invention relates to compositions comprising red yeast fermentation products, that can be used as dietary supplements and/or therapeutic medicaments. For example, the compositions can be used to lower serum cholesterol and triglycerides in mammals. Further, the invention relates to  
10 methods of treating cardiovascular disorders and other diseases using the red yeast fermentation products. In addition, the invention relates to particular *Monascus* strains that yield fermentation products with the desired biological activities.

15

**2. BACKGROUND**

**2.1 RED YEAST AND ITS USES**

Generally, red yeast (known in Chinese as Hung-ch'u or Hongqu) have been known and used for hundreds of years in  
20 China in rice wine making and as a food preservative. In addition, red yeast has been known for hundreds of years as an ancient Chinese medicine or an ingredient in certain ancient Chinese prescriptions; however, red yeast is most well known for its use in food as a preservative and  
25 colorant, and its uses in the dye industry.

Red yeast is a mixture of several species of *Monascus* fungi, notably *Monascus purpureus*. (Went, 1895, Ann Sci Nat Bot Ser, 8:1:1; Young, 1930, Trans Wisc Acad Sci Art Lett, 25:227-244). The genus of fungus, *Monascus* was first  
30 described in 1884 by van Tieghem (van Tieghem, 1884, Bull Soc Bot France, 31:226). The fungus was initially known to the Western world as a contaminant on cereals, starch and silage.

A detailed description of the medical applications of red yeast was provided in the ancient Chinese pharmacopoeia, Pen Ts'ao Kang Mu, which was published during the Ming  
35 dynasty (1368-1644). In Pen Ts'ao Kang Mu, which is still in print, red yeast is described as mild, non-poisonous, and useful for treating indigestion and diarrhea. Red yeast is

also described as useful for improving blood circulation, and promoting the health of the spleen and stomach. Furthermore, several "prescriptions" using red yeast for treating ailments, such as indigestion, diarrhea, and heart and abdominal pains, are also provided in this ancient work.

In an abbreviated English translation of Pen Ts'ao Kang Mu published in 1911, red yeast is described as useful for fermentation, and having medicinal value in the treatment of post-partum difficulties in women and dyspeptic conditions of children (Stuart, M.D., in "Chinese Materia Medica - Vegetable Kingdom", page 233-234, republished in 1979 by Southern Materials Center, Inc., Taipei, Republic of China). Red yeast, as described in Pen Ts'ao Kang Mu, was subsequently recognized to be the fungal species known as *Monascus purpureus* Went (Read, B.E., 1936, Chinese Medicinal Plants from the Pen Ts'ao Kang Mu, 3rd edition, published by Peking National History Bulletin; Klein, G., 1932, Handbuch der Pflanzenanalyse II, p. 1422-1423, Wien, Verlag von Julius Springer).

The manufacture of red yeast is taught in another publication from the Ming dynasty, T'ien Kung K'ai Wu by Sung Ying-Hsing, which was published in 1637 A.D (see pages 291-294 in English translation of this ancient writing, "T'ien Kung K'ai Wu - Chinese technology in the seventeenth century", translated by E-tu Zen Sun and Shiou-Chuan Sun, The Pennsylvania State University Press 1966). Red yeast is described therein as useful for preserving the color and taste of fish or meat. The manufacturing process used red wine mash and cooked non-glutinous rice as starting materials. The method of making red yeast by allowing the fungus to grow on the surface of cooked rice was also recorded by Voderman (1894, Analecta ob Cromatologisch Gebied. II. Geneesh. Fydschrift voor Ned. Indie, 35, No.5).

Nowadays, red yeast, which is still the fermentation product of *Monascus* species, is still used in traditional Chinese medicine, wine making and food coloring in Asia and Asian communities in North America. The red and yellow

pigments of *Monascus purpureus*, such as monascorubin and monascin, have been purified, and extensively studied (Fielding et al., 1961, J Chem Soc, 4579-4589). The culture conditions and its effect on pigmentation of *Monascus purpureus* have also been studied (Broder et al., 1980, J Food Sci, 45:567-469). Antibacterial activity, especially against *Bacillus* species, was also detected in *Monascus purpureus* extract (Wong, 1977, Plant Physiol, 60:578-581).

However, prior to the present invention, the extraordinary broad spectrum medicinal and nutritional benefits of red yeast in general, and certain species in particular, have not been thoroughly studied or appreciated.

## 2.2 HYPERLIPIDEMIA AND DIETARY INTERVENTION

Lipids and lipoproteins play an essential role in transporting fat-derived metabolites between organs for absorption, metabolism, and distribution (Felig et al., 1975, N Eng J Med, 293:1078-1084). The susceptibility to dietary-induced elevations in blood lipids including cholesterol is extremely common. The interaction of genetic predisposition and a high fat, high caloric diet coupled with underactivity can lead to heart disease, hypertension, hypertriglyceridemia, and diabetes in a significant proportion of the United States population.

High serum cholesterol is a major risk factor for coronary artery disease. Cholesterol is a major component of atherosclerotic plaque. Other associated lipid abnormalities, including hypertriglyceridemia especially in the presence of lowered HDL cholesterol levels, have been recognized as contributory to the risk of cardiovascular disease. There is a reciprocal relationship between elevated triglyceride levels and lowered HDL levels.

The level of cholesterol in circulation results from the balance between production of apoB-100 particles and its removal from the circulation. Cholesterol is synthesized from acetyl-CoA via a series of more than 20 enzymatic reactions. This biosynthetic pathway is mainly regulated by

the activity of HMG-CoA reductase (hydroxymethylglutaryl coenzyme A reductase), which catalyzes the reduction of HMG-CoA to mevalonate. Since the majority of cholesterol circulating is endogenously synthesized in the liver, and not  
5 derived from dietary cholesterol, inhibitors of enzymes that are involved in the biosynthesis of cholesterol have been explored as drugs for the treatment of hypercholesterolemia (Grundy 1988, New Eng J Med, 319:24-33).

A class of compounds inhibit cholesterol biosynthesis by  
10 competing with a natural substrate, HMG-CoA (hydroxymethylglutaryl coenzyme A), for the key enzyme in the cholesterol biosynthetic pathway, HMG-CoA reductase. The first such hypocholesterolemic compound discovered was compactin, which was isolated from cultures of *Penicillium*  
15 *citrinum* by Akira Endo (Endo et al., 1975, J Antibiotics, 29:1346-1348, see also U.S. patent nos. 3,983,140, 4,049,495, 4,137,322). The hypocholesterolemic activity of this compound was demonstrated in several animal species (Tsuji et al., 1979, Atherosclerosis, 32:307-313). Thereafter, a  
20 hypocholesterolemic compound structurally related to compactin was independently discovered by Endo in fermentation products of *Monascus ruber* (the active compound was named monacolin K; Endo, 1979, J Antibiotics, 32:852-854; Endo, 1980, J Antibiotics, 33:334-336; see also German  
25 patents DE 3051175, 3051099 and 3006216; British patent GB 2046737 and 2055100), and by another group from cultures of *Aspergillus terreus*. The active compound was also named mevinolin, lovastatin or Mevacor®; Tobert et al., 1982, J Clin Invest 69:913-919), and has been available in the United  
30 States since 1987 as a prescription drug. The efficacy and long term adverse effect of this active compound has been reviewed (Tobert, Am J Cardiol, 62:28J-34J). The isolated active compound, its derivatives and methods of production from *Aspergillus* have been reported; see U.S. patent nos.  
35 4,231,938, 4,342,767, 4,294,926, 4,319,039, 4,294,926, 4,294,846, and 4,420,491.



Although monacolin K or mevinolin has been successfully used to treat hypercholesterolemia, the compound has little or insignificant effect on the serum level of triglycerides. Other lipid regulating agents that have been used to treat  
5 hypertriglyceridemia, especially type IV and V hyperlipidemia, include nicotinic acid (e.g., niacin), and fibric acid derivatives (e.g., gemfibrozil and clofibrate). However, the uses of such agents are restricted because of their side effects, for example, high doses of niacin may  
10 causes gastric irritability, hyperuricemia, hyperglycemia, pruritus, and gemfibrozil may lead to malignancy, gallbladder diseases, and abdominal pain. Moreover, the risk of myositis and rhabdomyolysis that can result in renal failure increases when monacolin K is combined with gemfibrozil, clofibrate or  
15 niacin. Such combinations are only used with careful supervision in special situations that warrant the risk (The Merck Manual, 1922, 16th edition, pages 1044-1046). Since high concentrations of serum triglycerides are known to be a risk factor for a variety of disease states and it can lead  
20 to medical complications. Thus, there is a need for the development of a composition that accomplishes the reduction of the serum levels of both cholesterol as well as triglycerides.

Regular exercise and healthy nutrition achieving  
25 desirable body weights can prevent or reduce the incidence of common chronic diseases such as heart disease associated with elevations of blood lipids (1991, Pi-Sunyer, Am J Clin Nutr, 53:1595S-1603S). The role of diet in maintaining optimal health, and even in slowing and reversing the progression of  
30 disease or undesirable condition, has been the subject of much research and public attention. The development of an effective dietary supplement for use in the treatment of mixed hyperlipidemia would be a significant benefit to the health of Americans by improving the results that could be  
35 obtained with diet alone.

### 3. SUMMARY OF THE INVENTION

The invention relates to a product of the fermentation of at least one *Monascus* species that can be used as a dietary supplement or a therapeutic medicament to lower both serum cholesterol and triglyceride levels in humans. The invention is based, in part, on the surprising discovery that red yeast, i.e., the product of the fermentation of certain strains or mixtures of strains of *Monascus*, are effective at lowering not only the level of serum cholesterol but also the level of serum triglyceride in mammals, particularly humans. Since monacolin K or mevinolin are known not to be significantly effective in lowering serum triglyceride level, the beneficial effect of red yeast products must be related to other components in the fermentate.

In various embodiments of the invention, red yeast can be used as a natural dietary supplement or a medicament to treat or prevent a variety of diseases, including but not limited to cardiovascular diseases, diabetes, fatty liver conditions, stroke, cerebral thrombosis, hypotension, hypertension and obesity, and to modulate the circulating levels of lipids, such as cholesterol and triglyceride. In addition, the present invention encompasses methods for treating or preventing these diseases in a human, which comprise administering to the human a therapeutically effective amount of a red yeast fermentation product. The present invention also encompasses methods for improving or maintaining cardiovascular health in a human comprising administering to the human an effective amount of red yeast fermentation product. The present invention further encompasses methods for reducing the serum cholesterol and serum triglyceride levels to normal levels in a human comprising administering to the human a therapeutically effective amount of a red yeast fermentation product. Red yeast can also be used to treat or prevent a variety of ailments or symptoms as related to diseases of the cardiovascular system.



According to the invention, red yeast can be manufactured in various dosage forms and formulations. Also disclosed are methods for manufacturing red yeast which are based on the traditional fermentation procedures.

5

#### 4. DETAILED DESCRIPTION OF THE INVENTION

In one embodiment, the invention relates to compositions comprising the product of the fermentation of at least one *Monascus* species. These compositions are useful for reducing  
10 the levels of both serum cholesterol and serum triglycerides in mammals, and in particular humans. In addition, the compositions are useful for modulating the levels of both serum cholesterol and triglycerides to maintain healthy levels despite intrinsic (aging) or extrinsic (stress)  
15 factors that affect serum cholesterol and triglyceride levels.

The compositions and methods of the present invention are based, in part, on the discovery that the fermentate of *Monascus* species display hypocholesterolemic properties, and  
20 also unexpectedly, the ability to lower serum triglyceride levels. Since monacolin K is known not to be significantly effective in lowering serum triglyceride level, the beneficial effect of red yeast products must be related to other components of the fermentate. The ability of red yeast  
25 products to lower serum triglyceride level provides the art with a unique, natural alternative to the use of prescription hypocholesterolemic compounds.

The terms "red yeast fungi" or "*Monascus*" as used herein refer to the pre-fermented organism, while the terms "red  
30 yeast", "red yeast product", "red yeast extract" and the like refer to a product that results from the fermentation of at least one *Monascus*. Further, these latter terms include traditional and improved red yeast products as described below. More specifically, "red yeast product" as used herein  
35 refers to the product of fermentation, e.g., the fermentate, of one or a mixture of *Monascus* fungus.

Further, red yeast product is the fermentation product of at least one of the following *Monascus* fungi: *Monascus albidus*, *Monascus anka*, *Monascus araneous*, *Monascus aurantiacus*, *Monascus bakeri* Dangerd, *Monascus fuliginosus*,  
5 *Monascus kaoliang*, *Monascus major*, *Monascus paxii*, *Monascus pilosus*, *Monascus pubigerus*, *Monascus purpureus*, *Monascus ruber*, *Monascus rubiginosus*, *Monascus rubropunctatus*, and *Monascus serorubescens*.

In a preferred embodiment of the invention, red yeast is  
10 the fermentation product of a mixture of *Monascus* fungi, comprising chiefly *Monascus purpureus* Went, and in lesser proportions other *Monascus* species, e.g., preferably *Monascus ruber* van Tieghem, *Monascus Fuliginosus* Sato, *Monascus Pilosus* Sato and *Monascus albidus* Sato. In more preferred  
15 aspect, red yeast is the fermentation product of at least one of the following strains of *Monascus* fungi: *Monascus purpureus* Went ATCC 30141, AS 3.562, AS 3.991, AS 3.4446 [ATCC 16365], AS 3.4642 [NRRL 2897], AS 3.4643 [NRRL 96], AS 3.4644, AS 3.4645, AS 3.4651, *Monascus ruber* van Tieghem AS  
20 3.549, IFFI 05007, IFFI 05008, IFFI 05010, IFFI 05011, and *Monascus anka* IFFI 05038 (reference numbers provided in China Catalogue of Cultures, 1992, China Committee for Culture Collection of Microorganism, China Machine Press, Beijing 1992); and *Monascus purpureus* Went mutant strain M4027, 4028  
25 and M4184.

The term "traditional red yeast" as used herein refers to a red yeast product which is the result of fermentation using a mixture of *Monascus* fungi that has been used traditionally to manufacture red yeast. According to the  
30 invention, an "improved red yeast" is produced by fermentation using one or more natural or mutant strains of *Monascus* species, which yield a fermentate with an improved biological or nutritional properties, e.g., higher hypocholesterolemic and hypotriglyceridemic activities than  
35 traditional red yeast. Generally, the red yeast products of the present invention are red-purple powders that have a slightly bitter but mild and pleasant taste. Similarly, the

red yeast products have a pleasant odor. The color and/or odor may vary with the fermentation process, the strains used and the processing steps.

According to the invention, traditional or improved red yeast can be prepared by traditional fermentation procedures or by modification of the traditional procedures. According to the earliest reported method (Sung, 1637, T'ien Kung K'ai Wu; page 291-294, English translation by Sun et al., The Pennsylvania State Press 1966), red yeast can be prepared by the fermentation of washed and cooked non-glutinous rice using red wine mash, natural juice of Polygonum grass, and alum water. The rice is fermented in open air for 7 days on bamboo trays under very clean conditions. The rice changes its color from white to black, black to brown, brown to red and then red to yellow, which is then harvested as red yeast. According to an alternative traditional method, non-glutinous rice can be fermented in a hole in the ground lined by bamboo mats, which is securely covered. Fermentation is allowed to take place underground for one year or more, up to four years.

With respect to the present invention, the traditional method has been improved by use of modern fermentation techniques and equipment to more precisely control temperature, pH, pressure and other fermentation parameters, which, *inter alia*, reduces the time of fermentation. By way of an example, and not by limitation, improved red yeast of the invention can be prepared as follows: culture media containing kidney-bean juice 2%, sugar 4%, yeast 0.5% are added to rice (40 - 80 ml per 100g) and sterilized by heat, while the pH is maintained at pH3 to 8. Red yeast fungi *Monascus purpureus* Went strain M4184 is added and cultured at 15-35°C for 9 days. At the end of the fermentation process, the fermentation broth is drained and discarded, while the solid residue is sterilized by heat (for example, by high pressure steam), dried and crushed into powder. This powder can be used directly in the various compositions and formulations provided by the present invention.

Optionally, the dried crushed red yeast powder can be further processed, e.g., extracted with organic solvents, such as but not limited to, ethanol (75%) to remove starch and/or agar. After evaporation to dryness, the extract can  
5 be used in the various compositions and formulations as provided by the present invention. However, the isolation or purification of particular components of the product is not desired or contemplated by the present invention.

In various embodiments of the invention, red yeast can  
10 be used as a natural dietary supplement or a pharmaceutical medicament to maintain health or to treat or prevent a variety of diseases, including but not limited to cardiovascular diseases, diabetes, stroke, hypertension and obesity, and to modulate the circulating levels of lipids and  
15 lipoproteins, such as cholesterol and triglycerides. Red yeast can also be used to treat or prevent a variety of symptoms related to these above-mentioned diseases and associated with poor cardiovascular health due to aging and other intrinsic and extrinsic factors.

20 As used herein, examples of cardiovascular diseases, may include but are not limited to myocardial infarction, coronary heart disease, atherosclerosis, arteriosclerosis. The present invention includes the treatment or prevention of cerebrovascular disease such as stroke, memory loss due to  
25 stroke, and cerebral thrombosis.

The present invention also encompasses a composition comprising a therapeutically effective amount of a red yeast product, for example 2-4 grams per day, useful in humans for the treatment or prevention of hyperlipidemic disease,  
30 cardiovascular disease, cerebrovascular disease, hypertension, hypotension, diabetes, fatty liver conditions, or obesity, or a combination thereof.

The present invention further encompasses a composition comprising a therapeutically effective amount of a red yeast  
35 product, useful for the modulation of serum lipid and lipoprotein levels in a human in need of therapy to maintain the lipid and lipoprotein levels within a healthy normal

range. In one embodiment of the invention, the composition is adapted for use in the treatment or prevention of hypertriglyceridemia. In a preferred embodiment, such a composition is used for reducing serum cholesterol and serum  
5 triglyceride levels in humans.

The present invention further encompasses a composition comprising a therapeutically effective amount of improved red yeast product, useful for the treatment of any one of the following symptoms: shortness of breath, asthenic breathing,  
10 lethargy, dizziness, chronic headache, chest pain and tightness, heartache, loss of appetite, limb swelling, tightness and distention, and excess expectoration.

The phrase "therapeutically effective amount" as used herein means an amount sufficient to provide a therapeutic  
15 benefit in the treatment or prevention of the disease, or in the level of serum lipids and lipoproteins.

#### 4.1 METHODS OF TREATMENT

The present invention provides methods for treating a  
20 human afflicted by a variety of disease, disorder, and symptoms. In addition to treatment of a human disease, the methods of the invention can also be used for prevention in a human susceptible to such diseases, disorders or symptoms.

In one embodiment, the invention encompasses methods of  
25 treatment of hyperlipidemic disease, cardiovascular disease, cerebrovascular disease, hypertension (hereditary and non-hereditary), hypotension, angina, stroke, diabetes, fatty liver conditions, or obesity, or a combination thereof in a human, comprising administering to the human a  
30 therapeutically effective amount of a red yeast product, or compositions containing said product.

In another embodiment, the invention encompasses methods of preventing hyperlipidemic disease, cardiovascular disease, cerebrovascular disease, hypertension, hypotension, angina,  
35 stroke, diabetes, fatty liver conditions such as fatty liver deposits, obesity or a combination thereof, which comprises administering an effective amount of a red yeast product of



the present invention. In a preferred embodiment, the method of the invention is used to treat or prevent hypertriglyceridemia and associated diseases, such as hyperuricemia, pancreatitis and diabetes, in a human. As  
5 used herein, examples of cardiovascular diseases, may include myocardial infarction, coronary heart disease, atherosclerosis, arteriosclerosis; and cerebrovascular diseases or conditions, including stroke, cerebral thrombosis or memory loss due to stroke.

10 The present invention also provides methods for modulating serum lipid and lipoprotein levels in a human in need of lowering the lipid and lipoprotein levels to a healthy normal range, which comprise administering to the human a therapeutically effective amount of a red yeast  
15 product, or compositions containing said product. In a preferred embodiment, the method of the invention is used to reduce serum cholesterol and serum triglyceride levels in a human. The methods of the invention are particularly useful for the treatment of geriatric patients and post-menopausal  
20 women.

The present invention further provides methods for treating a human afflicted by shortness of breath, asthenic breathing, lethargy, dizziness, chronic headache, loss of appetite, limb swelling, tightness and distention, abdominal  
25 distention, and excess expectoration or a combination thereof, which comprises administering to the human a therapeutically effective amount of a red yeast product, or compositions containing said product.

The preventive or therapeutic dose of traditional red  
30 yeast or improved red yeast in the treatment or prevention of diseases and in the management of serum lipid and lipoprotein levels will vary with the condition to be treated and the severity of the condition to be treated. The dose, and perhaps the dose frequency, will also vary according to the  
35 age, body weight, and response of the individual patient. In general, the total daily dose range of red yeast, for the conditions described herein, is from about 0.1g to about 5g



administered in single or divided doses orally. For example, a preferred oral daily dose range should be from about 0.3g to about 4g, while most preferably an oral daily dose should be about 1.2 to about 2.5g. For example, two capsules each  
5 containing 0.6g of red yeast may be taken orally twice a day to obtain the preferred dose. A course of treatment should be at least 4 weeks. It may be necessary to use dosages outside these ranges in some cases as will be apparent to those skilled in the art. Further, it is noted that the  
10 nutritionist, dietician, clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with individual patient response.

It should be noted that the present invention encompasses new uses of traditional red yeast, and novel red  
15 yeast products and novel methods of using those products.

#### **4.1.1 USE AS A DIETARY SUPPLEMENT**

As mentioned above, the present invention encompasses compositions and methods of using traditional and novel or  
20 improved red yeast products as dietary supplements. As such, the red yeast products provide the individual with a means for maintaining normal or healthy levels of serum cholesterol and triglycerides despite intrinsic deterioration, e.g., from aging and extrinsic factors such as stress, lack of exercise  
25 and poor nutrition. The dietary supplements also provide a means for preventing, or reducing the likelihood of experiencing, the diseases discussed above. Finally, the dietary supplements can be used to prevent weight gain or obesity. Finally, the dietary supplements containing red  
30 yeast products are particularly useful for the elderly and post-menopausal women. The dietary supplements should be taken daily for at least four weeks and can be used permanently on a daily basis. A daily dose is from about 0.1g to about 5.0g; preferably about 1 to about 4g; and most  
35 preferably about 1.2 to about 2.4 grams per day.

#### 4.2 FORMULATIONS

The pharmaceutical and dietary compositions of the present invention comprise a red yeast product, or an extract thereof, as active ingredient, and may also contain a  
5 pharmaceutically acceptable carrier or excipient, and optionally, other ingredients.

Other ingredients that can be incorporated into the dietary or pharmaceutical compositions of the present invention, may include, but are not limited to, vitamins,  
10 amino acids, metal salts, and flavor enhancers. For oral administration, the compositions comprising red yeast can be added directly to foods so that a therapeutically effective amount of red yeast is ingested during normal meals. Any methods known to those skilled in the art may be used to add  
15 or incorporate red yeast to natural or processed foods.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, or tablets, each containing a predetermined amount of a red yeast product, as a powder or  
20 granules, or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid  
25 carriers or both, and then, if necessary, shaping the product into the desired presentation.

The compositions of the present invention may additionally include binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl  
30 methylcellulose); binders or fillers (e.g., lactose, pentosan, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate).  
35 The tablets or capsules can be coated by methods well known in the art.

Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid  
5 preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily  
10 esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also be made to resemble foods, containing buffer salts, flavoring, coloring and sweetening agents as appropriate.

15 Any dosage form may be employed for providing the patient with an effective dosage of the red yeast product. Dosage forms include tablets, capsules, dispersions, suspensions, solutions, capsules, and the like. Because of their ease of administration, tablets and capsules represent  
20 the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers as described above are employed. In addition to the common dosage forms set out above, the compounds of the present invention may also be administered by controlled release means. However, the most  
25 preferred oral solid preparations are capsules.

For example, a tablet may be prepared by compression or molding, optionally, with one more accessory ingredients. Compressed tablets may be prepared by compressing in a  
30 suitable machine red yeast in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Most preferably, the composition is a capsule containing 0.3g of red yeast in powder form.

The invention is further defined by reference to the  
35 following examples describing in detail the human clinical trials conducted to study the efficacy and safety of red yeast. It will be apparent to those skilled in the art that

many modifications, both to materials and methods, may be practiced which are within the scope of this invention.

## 5. PHARMACOLOGY AND TOXICOLOGY

5        Pharmacological and toxicological studies of red yeast  
of the present invention were performed in experimental  
animal models. Red yeast were shown to dramatically decrease  
serum total cholesterol (TC) of endogenous hyperlipidemic  
rabbits; remarkably decrease serum TC and total triglyceride  
10 (TG) of exogenous hyperlipidemic rabbits; inhibit formation  
of arteriosclerosis plaque and lipid deposition in liver in  
hyperlipidemic rabbits; and decrease serum TC and TG of  
hyperlipidemic quails.

In acute toxicity studies, a LD<sub>50</sub> value cannot be  
15 determined. The highest tolerance dose of red yeast in mice  
is over 16g/kg, which is 533 times over the dose used in  
clinical treatment. Moreover, in other experiments, rats  
were continuously force-fed red yeast for four months; no  
rats died or showed toxic symptoms due to this drug.

20 Hematological indices, main viscera indices, blood biological indices, routine uroscopy and pathological examination did not show any differences between experimental groups and control groups.

25

The following sections contain the methodologies and results of two human clinical trials that were carried out in China. The trials were aimed to determine the efficacy of a red yeast product in modulating circulating serum lipid and lipoprotein levels in humans, in resolving symptoms according to traditional Chinese medicine, and in establishing the safety of a red yeast product.

## 6.1 CLINICAL TRIAL I

35 In this randomized human clinical trial, 446 patients with hyperlipidemia, who were also diagnosed as suffering from hypofunction and disorder of the spleen and excess

expectoration by traditional Chinese medicine, were divided into two treatment groups. One group having 324 patients received Xuezhikang capsule, and the other control group having 122 patients received Jiaogulan tablets.

- 5 Xuezhikang capsule contains 0.3g of a red yeast product. Jiaogulan tablet is a lipid-regulating drug (*Gynostemma pentaphyllum*) that is based on traditional Chinese herbal medicine.

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#### 6.1.1 METHODOLOGY

##### 6.1.1.1 CRITERIA FOR PATIENT SELECTION

All the patients with primary hyperlipidemia stopped using serum lipid modulators two to four weeks prior to the beginning of the trial and received dietary advice. Serum  
15 sample was taken and laboratory test was conducted. Only patients who met the following criteria were enrolled in the trial: total serum cholesterol (TC) > 230 mg/dl (5.95 mmol/L) and triglyceride (TG) > 200 mg/dl (> 2.26 mmol/L/L). High density lipoprotein cholesterol (HDL-C) was also considered  
20 as a reference: male < 40 mg/dl (1.04 mmol/L), female < 45 mg/dl (1.16 mmol/L). All patients were diagnosed as deficient in the function of the spleen and having excess expectoration by traditional Chinese medicine. The patients also had the following symptoms: limb weakness, asthenic  
25 breathing, pain and oppressed feeling in chest, loss of appetite, distention and swelling on gastric region, whitish or purple dots on the tongue, the thick-white or thick-slimy fur on the tongue, taut-slippery or hesitant-weak pulse.

Patients who had the following disorder or disease were  
30 excluded from the trial: myocardial infarction, cerebrovascular disease, severe wound or major surgery during the past half year, nephritic syndrome, hypothyroidism, acute and/or chronic hepatobiliary disorder, diabetes, gout, general allergic reactions, and psychosis.

- 35 The total number of patients enrolled was 446. In the treated group, there were 188 male and 126 female patients. The ratio of male versus female was 1.38:1 and the average

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## 25

1. Cure: All symptoms were eliminated, or a reduction of the total symptom score by more than 90%, and a return of all laboratory test parameters to normal.
2. Effective: Symptoms were significantly relieved, i.e., symptom score reduced by 70%-89%. Serum lipid and lipoprotein did not reach normal, but were improved in one of the following respects: 1) reducing TC  $\geq$  20%, 2) reducing TG



$\geq 40\%$ , 3) reducing  $(TC-HDL-C)/HDL-C \geq 20\%$ , 4) increasing  $HDL-C > 10 \text{ mg/dl}$ .

3. Improvement: Symptoms were relieved, i.e., symptom score reduced by 30%-69%. Serum lipid and lipoprotein levels were not normal but were improved in one of the following respects: 1) reducing TC at 10%-20%, 2) reducing TG  $\geq 20\%$  but  $< 40\%$ , 3) reducing  $(TC-HDL-C)/HDL-C \geq 10\%$  but  $< 20\%$ , 4) increasing  $HDL-C > 4 \text{ mg/dl}$  ( $0.14 \text{ mmol/L}$  but  $< 10 \text{ mg/dl}$ ).

4. Inefficacy: Symptom score was reduced by less than 30%, and the laboratory test parameters did not meet the criteria of effectiveness.

All the data are subjected to statistical analysis. Student test was used in measuring data, Chi-square test for counting data, Ridit assay for data of stratum and U chart for percentiles analysis.

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Table - 1: Efficacy comparison

	Case number	Cure		Effective		Improvement		Inefficacy		Total Effective		Improvement	
		n	%	n	%	n	%	n	%			n	%
Treated Group	324	169	52.2	89	27.5	44	13.5	22	6.8	258	79.7	302	93.2
Control Group	122	13	10.7	25	20.5	24	19.7	60	49.2	38	31.1	62	50.8

Ridit Analysis: u=10.04, p<0.001

Table - 2: Comparison of serum lipid and lipoprotein levels after treatment

Parameter	Group	Case No.	Mean±S Baseline mg/dl	Difference after 4 week difference in mg/dl	% Change	Difference after 8 week difference in mg/dl	% Change
TC	Treated	251	273.5±31.3	-47.4	-17.3**	-62.8	-23**
	Control	94	268.2±25.4	-13.2	-4.9**	-18.9	-7**
TG	Treated	183	296.0±75.5	-66.3	-22.4**	-108	-36.5**
	Control	72	289±71.7	-27.5	-9.5*	-42.3	-14.6**
HDL-C	Treated	121	35.9±4.4	4.2	11.8**	7	19.6**
	Control	55	35.1±4.0	1.8	5*	3	8.6**
LDL-C	Treated	324	162.2±52.4	-36.5	-22.5**	-46.3	-28.5**
	Control	122	157.3±49.2	-9	-5.7**	-12.6	-8**
TC-HDL-C/ HDL-C	Treated	324	4.69±1.44	-1.3	-27.7**	01.6	-34.2**
	Control	122	4.79±1.71	-0.39	-8.1**	-0.52	-10.9**

Note: (+) indicates increase, (-) indicates decrease  
 \*:p<0.01, \*\*:p<0.001 vs. baseline  
 +:p<0.05; ++:p<0.01; +++:p<0.001 vs. control

### 6.1.2 RESULTS

Table 1 shows a comparison of overall efficacy wherein the score for the treated group was much higher than that in the control group ( $X^2=9.7$ ,  $P<0.001$ ).

5        The percentage of patients in the treated group, who reported the elimination of symptoms diagnosed by traditional Chinese medicine, was much higher than that in the control group ( $p<0.05$  -  $0.001$ ). Those symptoms were: condition of tongue (whitish or purple dots on the tongue; thick-slimy  
10 fur); pulse (slippery-taut or hesitant-weak), oppressed feeling in chest; loss of appetite; abdominal distention and swelling.

With respect to serum lipid and lipoprotein level, the efficacy scores for curing or reducing total serum  
15 cholesterol and total triglyceride level in the treated group were greater than that in the control group. The score for normalizing or increasing HDL-C, level and the score for reducing Atherosclerotic Index in the treated group were also much better than the control ( $P<0.001$ ).

20        Table 2 indicates that both Xuezhikang-treated and control groups showed marked desirable changes in the levels of TC, TG,  $(TC - HDL-C)/HDL-C$ , HDL-C serum levels markedly. The effectiveness of Xuezhikang was found to be superior to that of Jiaogulan.

25        It was also observed that the higher the baseline of TC and TG in the serum, the more effective is the reduction of TC and TG after using Xuezhikang. As for HDL-C level, a greater increase was observed after treatment in patients who had a lower starting baseline.

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Table - 3: Effects of Xuezhikang capsule on patients with different abnormal levels serum lipid and lipoprotein.

	TC (mg/g)			TG (mg/g)			HDL-C (mg/dl)		
Parameter	<230	230-300	>300	<230	230-300	>300	>45	35-45	<35
Case No.	73	206	45	141	112	71	161	114	49
Mean baseline (mean)	187.8	261.8	327.1	134.3	247.6	327.3	56.4	40.1	5.4
Difference (4 weeks)	↓20.5	↓42.5	↓69.8	↓2.7	↓51.4	↓89.8	↓1.3	↓4	↓5.4
% Changes	↓10.9	↓16.2	↓21.3	↓2	↓20.8	↓24.1	↓2.3	↓10	↓17
Difference (8 weeks)	↓30.6	↓57.9	↓86.1	↓15.9	↓81.4	↓149.9	↓2.1	↓6.3	↓7.2
% Changes	↓16.3	↓22.1	↓26.3	↓11.8	↓32.9	↓40.2	↓3.7	↓15.7	↓22.8
Comparison	**	**	**	**	**	**	*	*	*

(↓) indicates the value increase  
(↓) indicates the value decrease  
\* P<0.01; \*\* P<0.001 vs baseline

Table - 4: Effect of Xuezhikang capsule on apoA-I and apoB (mean±S)

Group	Case No.	Time Point	apoA-I	apoB	apoA-I/apoB
Treated Group	88	Baseline	1.22±0.19	1.2±0.19	1.05±0.25
		4 Weeks	1.32±0.13 (4) ↑8.2%	1.09±0.21 (3) ↓9.2%	1.25±0.27 (5) ↑19%
		8 Weeks	1.28±0.13 ↑4.9%	0.99±0.18 (3) ↓18%	1.33±0.30 (3) ↑26.7%
Comparison Group	30	Baseline	1.19±0.16	1.21±0.15	1.00±0.18
		4 Weeks	1.26±0.11 (1) ↑5.9%	1.15±0.17 (1) ↓5%	1.11±0.14 (2) ↑11.0%
		8 Weeks	1.26±0.09 (1) ↑5.9%	1.03±0.15 (3) ↓14.9%	1.24±0.21 (3) ↑24.0%

(1) P<0.05; (2) P<0.01; (3) P<0.001; vs. baseline  
(4) P<0.05; (5) P<0.05; vs. control



Regarding the effect of Xuezhikang on apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB), the serum levels of apoA-I in both groups were raised after therapy. Statistical results show a significant difference in apoA-I levels after  
5 a four week treatment. ApoB levels were reduced somewhat in both groups, however, these reductions are not statistically significant. The treated group showed better improvement of apolipoprotein B and apoA-I/apoB over the control.

With respect to rheology, there were significant changes  
10 in blood sedimentation and K-value in both groups after treatment ( $P < 0.05-0.01$ ). However, the treated group showed better results than the control group ( $P < 0.05-0.01$ ).

All 446 patients were subjected to the following laboratory tests before and after therapy: blood urea  
15 nitrogen (BUN), creatinine, serum glutamic pyruvic transaminase (SGPT, ALT), serum glucose, and creatinine kinase (CK), and routine examination of blood and urine. No clinically meaningful changes were found at the end of the trial.

20 Several patients developed a burning sensation in the stomach (six patients, 1.8%), experienced fullness in the stomach (three patients, 0.9%), and suffered dizziness (one patient 0.3%). All had finished the trial, and all the symptoms were spontaneously relieved without treatment. Two  
25 patients suffered gastritis after taking Xuezhikang and had to leave the trial. All the results suggest that Xuezhikang is a safe drug.

#### 6.1.3 DISCUSSION

30 In this trial, a lipid regulating agent known in traditional Chinese medicine was used as a positive control. Between the two groups, there were no difference ( $P > 0.05$ ) found in baseline parameters including age, sex and course of disease, serum lipid and lipoprotein levels. The efficacy  
35 score in the Xuezhikang-treated group was much higher than that in the control group ( $P < 0.001$ ). Comparing the baseline, in Xuezhikang-treated group, serum level of high density

lipoprotein cholesterol was elevated by 19.6% and total cholesterol, total triglyceride, low density lipoprotein cholesterol and Atherosclerosis Index were reduced by 23%, 36.5%, 28.5% and 34.2% respectively. It was also observed  
5 that the higher the abnormality of the lipid and lipoprotein serum level, the more dramatic the modulation of lipid and lipoprotein levels can be achieved by Xuezhikang therapy. Xuezhikang can also reduce apolipoprotein B level, blood sedimentation and blood sedimentation K-value.

10 Overall, the results show that red yeast is a safe, effective agent for modulating serum lipid and lipoprotein levels. Red yeast can also be used as a therapeutic agent for coronary artery disease and cerebrovascular disease caused by hyperlipidemia and/or atherosclerosis because red yeast  
15 not only significantly reduced plasma TC, TG and Atherosclerosis Index, but also markedly raised plasma apolipoprotein A-I level.

## 6.2 CLINICAL TRIAL II

20 In this clinical trial, 84 patients with hyperlipidemia, and 56 patients who were also diagnosed with atherosclerosis were divided into two treatment groups: a group treated with Xuezhikang capsule and a control group treated with Jiaogulan tablet.

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### 6.2.1 METHODOLOGY

#### 6.2.1.1 CRITERIA FOR PATIENT SELECTION

All patients were diagnosed as hyperlipidemic following the criteria set forth in "Clinical trial management:  
30 hyperlipidemia treatment using new Chinese materia medica" released by the Ministry of Health of China. After dietary advice for two to four weeks, patients with abnormal lipid and lipoprotein were bled twice two weeks prior to the trial. Only patients who met the following criteria were enrolled in  
35 the trial: total serum cholesterol (TC) > 230 mg/dl (5.95 mmol/L) and triglyceride (TG) > 200 mg/dl (> 2.26 mmol/L). High density lipoprotein cholesterol (HDL-C) was also considered

as a reference: male < 40 mg/dl (1.04 mmol/L), female < 45 mg/dl (1.16 mmol/L).

Patients who had stagnation of phlegm caused by deficiency in the function of the spleen were also selected.

- 5 The symptoms included: limbs tightness, asthenic breathing, pain and oppressed feeling in chest, loss of appetite, distention and swelling on gastric region, whitish or purple dots on tongue, the thick-white or thick-slimy fur on tongue, taut-slippery or hesitant-weak pulse.

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#### 6.2.1.2 SCORE FOR CLINICAL SYMPTOMS

The severity of the symptoms as recognized by traditional Chinese Medicine were scored as follows:

##### Asthenic breathing

15	0	none	(-)	No asthenic breathing
	2	light	(+)	having asthenic breathing with physical activity
	3	moderate	(++)	having medium asthenic breathing with physical activity
20	4	severe	(+++)	having asthenic breathing at rest

##### Limbs tightness

25	0	none	(-)	no Limbs tightness
	2	light	(+)	having Limbs tightness occasionally
	3	moderate	(++)	having medium Limbs tightness very often
30	4	severe	(+++)	having severe Limbs tightness

##### Chest tightness and pain

35	0	none	(-)	no Chest tightness and pain
	2	light	(+)	having Chest tightness and pain occasionally

	3	moderate	(++)	having medium Chest tightness and pain very often
	4	severe	(+++)	having severe Chest tightness and pain at rest
5				
		Loss of appetite		
	0	none	(-)	having normal appetite
	2	light	(+)	losing appetite by 1/4 - 1/3
10	3	moderate	(++)	losing appetite by 1/3 - 1/2
	4	severe	(+++)	losing appetite more than 1/2
		Abdominal distention and swelling		
15	0	none	(-)	No this sign
	2	light	(+)	having the sign occasionally
	3	moderate	(++)	having the sign very often
	4	severe	(+++)	having severe abdominal distention and swelling
20				
		Picture of the tongue		
	0	normal	(-)	
	1	abnormal	(+)	
		Pulse condition		
25	0	normal	(-)	
	1	abnormal	(+)	
	Symptom severity: light: score $\leq$ 12			
	moderate score 12 - 20			
	Severe score > 20			

30 Patients diagnosed by traditional Chinese medicine according to the above symptoms, and patients with primary hyperlipidemia were enrolled.

The criteria for exclusion of patients were as follows:

- a: myocardial infarction, cerebrovascular disease,  
 35 severe wound or major surgery during last half year;  
 b: nephritic syndrome, hypothyroidism, acute and/or chronic hepatobiliary disorder, diabetes, gout;

c: familial hypercholesterolemia (monogenic-hypercholesterolemia);

d: secondary hyperlipidemia caused by other medication, for instance: phenothiazine, beta-adrenergic blocking agents, corticosteroid, oral contraceptive;

e: patients who used other lipid modulators during the last four weeks and patients using heparin or were on thyroidization;

f: pregnant and breast-feeding women;

10 g: patients with disorder of the other organs; and

h: hylaxis syndrome, and psychosis.

The total number of patients enrolled was 116. There were 84 patients in the treated group and 32 patients in the control group. No difference of distribution in age, sex and course of disease were found between the two groups.

#### 6.2.1.3 TREATMENT PROTOCOL

A randomized single-blind trial was conducted with two groups. The treated group (84 cases) took two Xuezhikang capsules (i.e., a red yeast product of the present invention) twice a day. The control group (32 cases) took three Jiaogulan tablets (ShanXi factory of Chinese material medica, lot number: 940730) twice a day. The course of treatment was eight weeks.

25 The measurements of serum lipid and lipoprotein levels and other scoring were performed prior to the therapy, and at four weeks and at eight weeks after therapy. The safety tests were conducted before and after therapy. Venous blood samples were taken from patients before breakfast, who were not allowed to consume alcohol or food with a high fat content in the last meal.

The following safety tests were conducted: blood and urea nitrogen (BUN), creatinine, serum glutamic pyruvic transaminase (SGPT, ALT), serum glucose, and creatinine kinase (CK). Total serum cholesterol (TC), total serum triglyceride (TG) and high density lipoprotein cholesterol levels were measured to determine efficacy. Other relevant

clinical sign such as, weight, high blood pressure heart beat, and rhythm, and hepatospleno-palpation were recorded.

#### 6.2.1.4 EFFICACY

5       Efficacy was evaluated according to the criteria set forth in "Clinical trial management: hyperlipidemia treatment using new Chinese materia medica" released by the Ministry of Health of China as follows:

- 10       1. Cure: All symptoms are eliminated or the total symptom score reduced by more than 90%, and every laboratory tested parameters reached normal levels.
- 15       2. Effective: Symptoms are significantly relieved, i.e., symptom score reduced by 70%-89%. Serum lipid and lipoprotein do not reach normal level but was improved in one of the following respects: 1) reducing TC  $\geq$  20%, 2) reducing TG  $\geq$  40%, 3) reducing (TC-HDL-C)/HDL-C  $\geq$  20%, 4) increasing HDL-C > 10 mg/dl
- 20       3. Improvement: Symptoms are relieved, i.e., symptom score reduced by 30%-69%. Serum lipid and lipoprotein did not reach normal levels but were improved in one of the following respects: 1) reducing, TC at 10%-20%, 2) reducing TG  $\geq$  20% but < 40%, 3) reducing (TC-HDL-C)/HDL-C  $\geq$  10% but < 20%, 4) increasing HDL-C > 4 mg/dl (0.14 mmol/L).
- 25       4. Inefficacy: Symptom score was reduced by less than 30% and laboratory test parameters did not meet the criteria of effectiveness.

All the data were subjected to statistical analysis. Student t test was used in measuring data, Chi-square test for counting data, and Ridit assay for data of stratum.

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#### 6.2.2 RESULTS

Table 5 shows a comparison of the overall efficacy score of the two groups.

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Table 5 General Efficacy

	Group	Total Case Number	Cure	Effective	Improvement	Inefficac y
5	Treated	84	39	25	13	7
	Control	32	3	6	4	19

Ridit Analysis:  $u=5.18$ ,  $P<0.01$

Comparison:  $X^2=0.0$   $P>0.05$

10        The total efficacy score in the treated group was much higher than that of the control group ( $X^2=22.95$ ,  $P<0.01$ ).

Table 6 shows a comparison of efficacies as defined by the standards of traditional Chinese medicine.

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Table 6 Comparison of efficacy

Symptoms	Red Yeast			Control			Statistics
	Before	After	Vanish %	Before	After	Vanish %	
Asthenic breathing	35	15	57.1	12	6	50	>0.05
Limbs tight	37	16	56.8	13	7	46.2	>0.05
Oppressed feeling in chest	37	16	56.8	9	5	44.4	*
Chest pain	8	1	87.5	2	1	50	*
Lose of appetite	11	4	63.6	3	2	33.3	*
Distension & swelling stomach	31	11	64.5	7	6	14.3	*
Pale tongue	54	34	37	17	12	31.3	>0.05
Purple dot on tongue	9	4	55.6	3	4	0	*
Thick-whitish fur	21	16	23.8	11	6	45.5	>0.05
Thick-slimy fur	11	8	27.3	5	4	0	
Slippery & string-like pulse	28	22	21.4	13	8	33.3	*
Weak-thread pulse	24	16	33.3	8	3	62.5	*
Slippery-fine pulse	28	16	42.9	9	8	11.1	*

\*P&lt;0.05 vs control

The percentage of patients who reported elimination of the symptoms as diagnosed by traditional Chinese medicine in the treated group was much higher than that in the control group ( $p < 0.05$ ), especially in the aspect of pain and oppressed feeling in chest, loosing appetite, distention and swelling on gastric region as well as purplish dots on the tongue.

The change in serum total cholesterol level is shown in Table 7.

10 Table 7: Change in serum total cholesterol level

Group	Abnormal Case No.	Cure	Reduction >20%	Reduction 10-20%	Reduction <10%
Treated	76	53	9	5	9
Control	28	4	0	2	22

Ridit Analysis:  $u=5.47$   $P>0.05$

Efficacy ratio:  $X^2 = 39.96$ ,  $P<0.001$ , vs. control

The scores for curing or reducing total serum cholesterol level in the treated group were greater than that in the control group.

The change in serum total triglyceride level is shown in Table 8.

25 Table 8 Change in serum total triglyceride level

Group	Abnormal Case No.	Cure	Reduction >20%	Reduction 10-20%	Reduction <10%
Treated	35	20	2	5	9
Control	13	6	2	1	4

30 Ridit Analysis:  $u=0.53$   $P>0.05$

Efficacy ratio:  $X^2 = 0.007$ ,  $P<0.05$ , vs. control

No significant difference in the scores for curing or reducing the serum total triglyceride level was observed between the two groups.

The change in high density lipoprotein-cholesterol level is shown in Table 9.

Table 9 Change in HDL-C levels.

Group	Abnormal (>4) Case No.	Cure >4	Reduction >20%	Reduction 10-20%	Reduction <10%
5 Treated	24	11	1	3	9
Control	10	3	0	1	6

Ridit Analysis:  $u=1.03$   $P>0.05$

Efficacy ratio:  $X^2 = 1.15$ ,  $P<0.05$ , vs. control

- 10 A similar efficacy for normalizing or increasing HDL-C level was found in both groups.

Table 10 shows the changes in Atherosclerotic Index which is the ratio of (TC-HDL-C)/HDL-C.

15 Table 10 Change of (TC-HDL-C)/HDL-C

Group	Abnormal (>4) Case No.	Cure >4	Reduction >20%	Reduction 10-20%	Reduction <10%
20 Treated	56	44	6	3	3
Control	14	3	1	2	8

Ridit Analysis:  $u=3.84$   $P>0.01$

Efficacy ratio:  $X^2 = 23.41$ ,  $P<0.01$ , vs. control

- 25 The data in Table 10 indicates the Xuezhikang-treated and control groups improved HDL-C serum levels markedly. The effectiveness of Xuezhikang was found to be superior to that of the control.

Table 11 shows the effect of Xuezhikang on regulating  
30 serum lipid and lipoprotein levels ( $X\pm S$ ).

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Table 11: Regulating Effect of Xuezhikang on serum lipid and lipoprotein (X±S)

Group	Time Point	Case No.	TC (mg/dl)	Case No.	TG (mg/dl)	Case No.	HDL-c (mg/dl)	Case No.	LDL-c (mg/dl)
Treated Group	Baseline	76	273.9±34.1	35	304.1±86.8	24	35.3±4.8	84	174.7±48.1
	4 Weeks difference		253.9±35.9 35*** ↓13.87%		270.3±121.2* 33.8 ↓11.11%		39.5±9** 4.2# ↑11.9%		141.4±477.6*** 33.3### ↓19.1%
	8 Weeks difference		216.7±33.7 57.3*** ↓20.91%		206.5±72*** 97.6 ↓32.09%		42.1±7.6*** 6.8 ↑19.6%		126.8±39.6*** 49.9### ↓27.4%
Control Group	Baseline	28	265.4±25	13	297.1±72.1	10	35.3±3.3	32	164.3±35.6
	4 Weeks difference		272.6±33.3 7.2 ↑2.7%		304.8±0.141 7.7 ↑2.6%		35.3±3.7 0.02 ↓0.06%		171.6±42.3 7.3 ↑4.44%
	8 Weeks difference		265±35.8 0.5 ↓0.2%		226.7±88.1* 70.3 ↓23.67%		38.3±7.3 2.98 ↑8.43%		168±45.5 4.11 ↑2.5%

↑: Increase    \*\*\* P<0.001; \*\*P<0.01; \*P<0.05 vs baseline  
↓: Decrease    ##P<0.05) P<0.05 vs. control

Table 11 indicates that Xuezhikang improved TC, TG, (TC - HDL-C)/HDL-C, HDL-C serum levels markedly, but control group only improved TG significantly. The ability to regulate TC, LDL-C of Xuezhikang therapy was found to be superior to the control.

Table 12 shows the efficacy of Xuezhikang therapy on different baseline of serum lipid and lipoprotein

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Table - 12: Efficacy comparison of Xuezhikang therapy on different baseline of serum lipid and lipoprotein.

	TC (mg/g)			TG (mg/g)			HDL-C (mg/dl)		
Parameter	<230	230-300	>300	230	230-300	>300	>45	35-45	<35
Case No.	8	60	16	49	20	15	55	17	12
Mean baseline (mean)	192.01	261.8	327.1	134.3	247.6	327.3	56.4	40.1	5.4
Mean (4 weeks)	174.14	226.14	272.56	134.84	202.64	360.59	55.03	43.41	38.82
% Changes	↓9.31	↓12.621	↓17.62	↓4.05	↓16.2	↓6.87	↓2.68	↑7.49	↑24.05
Mean (8 weeks)	156.08	208.21	↓86.1	119.38	169.51	255.88	57.58	46.89	40.33
% Changes	↓18.72	↓19.53	↓24.93	↓15.05	↓29.9	↓33.91	↑1.85	↑16.11	↑28.89
Comparison	*			**			**		

\*\* P<0.01; \* P<0.001 vs. control



The Higher baseline of TC and TG in the serum, the more reduction is achieved after using Xuezhikang.

### 6.2.3 DISCUSSION

5       The results indicate that the score of cure, and the score of efficacy were 46.4% (38/84), 29.8% (25/84) respectively in the Xuezhikang-treated (red yeast treated) group and 9.4%(3/32), 18.8 (6/32) in the control group. Total efficacy ratio in the treated group (72%) was much  
10 higher than that in the control group (28.2%,  $P < 0.001$ ).

There were significant differences between the two groups in terms of improving TC, LDL-C and (TC - HDL-C)/HDL-C, but no difference was found in terms of regulating TG and HDL-C even the Xuezhikang group showed  
15 better results.

No significant clinically meaningful change in the following parameters was observed during and after therapy: serum glutamic pyruvic transaminase (SGPT), blood and urea nitrogen (BUN), creatinine, serum glucose, cardioelectrogram,  
20 and routine examination of urine and blood. Three cases reported an increase in creatinine kinase (CK) (252, 260, 466 IU/L versus normal standard at 200IU/L) in the treat group and one (256 IU/L) in the control group. No clinical symptoms were observed in any of these cases. The results  
25 show that a red yeast product of the present invention is a safe and acceptable lipid-modulating agent.

It may be apparent to those skilled in the art that modifications and variations of the present invention are possible in light of the above disclosure. It is understood  
30 that such modifications are within the spirit and scope of the invention, which is defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method of treating or preventing a cardiovascular disorders in a human which comprises administering to a human  
5 an effective amount of a red yeast fermentation product.

2. The method of claim 1 wherein said red yeast fermentation product is produced from the fermentation of at least one *Monascus* species.

10

3. The method of claim 1 wherein said cardiovascular disorder is selected from the group consisting of myocardial infarction, coronary heart disease, hypertension, hypotension, atherosclerosis and arteriosclerosis.

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4. A method of treating or preventing stroke, cerebral thrombosis or memory loss due to stroke in a human which comprises administering to a human an effective amount of a red yeast fermentation product.

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5. A method of treating or preventing fatty liver conditions in a human which comprises administering to a human an effective amount of a red yeast fermentation product.

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6. The method of claim 4 or 5 wherein said red yeast fermentation product is produced from the fermentation of at least one *Monascus* species.

30

7. A method of improving or maintaining cardiovascular health which comprises administering to a human an effective amount of a red yeast fermentation product.

8. The method of claim 7 wherein said red yeast  
35 fermentation product is produced from the fermentation of at least one *Monascus* species.

9. A method of treating or preventing diabetes in a human which comprises administering to a human an effective amount of a red yeast fermentation product.

5 10. The method of claim 9 wherein said red yeast fermentation product is produced from the fermentation of at least one *Monascus* species.

11. A method of treating or preventing obesity in a  
10 human which comprises administering to a human an effective amount of a red yeast fermentation product.

12. The method of claim 11 wherein said red yeast fermentation product is produced from the fermentation of at  
15 least one *Monascus* species.

13. A method of reducing serum cholesterol and triglyceride levels to normal levels in a human in need of such therapy which comprises administering an effective  
20 amount of a red yeast fermentation product.

14. The method of claims 1, 4, 5, 7, 9 or 11 wherein said administration is made once or twice daily and for a period of time sufficient to treat or prevent said disorder.  
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15. The method of claims 1, 4, 5, 7, 9 or 11 wherein said red yeast fermentation product is produced from the fermentation of a mixture of *Monascus purpureus* Went, *Monascus ruber van Tieghem*, *Monascus Fuliginosus* Sato,  
30 *Monascus Pilosus* Sato, and *Monascus albidus* Sato.

16. The method of claims 1, 4, 5, 7, 9 or 11 wherein said red yeast fermentation product is produced from the fermentation of a *Monascus purpureus* Went strain.  
35

17. The method of claims 1, 4, 5, 7, 9 or 11 wherein said effective amount is about 1 to about 4 grams per day.

18. A pharmaceutical or nutritional composition suitable for oral or parenteral administration to a human which comprises an effective amount of a red yeast product.

5        19. The composition of claim 18 wherein said composition is suitable for oral administration as a capsule, tablet or cachet.

20. The composition of claim 18 wherein said  
10 composition is suitable for oral or parenteral administration as a suspension, solution or other suitable liquid delivery system.

21. The composition of claim 18 which further comprises  
15 an excipient or carrier.

22. The composition of claim 18 which further comprises at least one vitamin supplement or flavor enhancer.

20        23. A medicament which comprises about 600mg of a red yeast fermentation product.

24. The composition of claims 18 or 23 wherein said red yeast fermentation product is produced from the fermentation  
25 of at least one *Monascus* species.

25. The composition of claims 18 or 23 wherein said red yeast fermentation product is produced from the fermentation of a mixture of *Monascus purpureus* Went and at least one  
30 other *Monascus* strain.

26. The composition of claims 18 or 23 wherein said red yeast fermentation product is produced from the fermentation of a *Monascus purpureus* Went strain M4027, M4028 or M4184.  
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27. A red yeast fermentation product suitable for oral delivery to humans which comprises: (a) fermenting at least

one species of *Monascus purpureus* Went and non-glutinous rice; (b) draining the resulting fermentation broth; and (c) sterilizing the product with heat.

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/17574

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 9/20, 35/00, 35/72

US CL : 424/93.50, 93.51, 115, 464, 514/824, 909

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.50, 93.51, 115, 464, 514/824, 909

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 4,361,515 A (TERAHARA et al) 30 November 1982, column 1, lines 7-15 and 29-31, column 47, lines 56-64, and column 48, lines 4-37.	1-3, 7, 8, 14, 18, 19, 24 ----- 5, 6, 13, 15-17, 20-23, 25, 26
Y	ZHU. Y. et al. Effects of Xuezhikang on blood lipids and lipoprotein concentrations of rabbits and quails with hyperlipidaemia. Chinese Pharmaceutical Journal. 1995. Vol. 30. No. 11. English translation of abstract only.	1-3, 5-26



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

24 NOVEMBER 1997

Date of mailing of the international search report

19 DEC 1997

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/17574

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KOHAMA. Y. et al. Isolation and identification of hypotensive principles in red-mold rice. Chem. Pharm. Bull. 1987. Vol. 35. No. 6. pages 2484-2489, especially page 2484, introduction, page 2485, Table 1, and page 2488, discussion.	1-3, 5-8, 13-27
Y	KUSHIRO. T. et al. Clinical effects of beni-koji in mild essential hypertension--a multi-center double-blind comparison with placebo. Nippon Jinzo Gakkai Shi, Japanese Journal of Nephrology. December 1996. Vol. 38. No. 12. pages 625-633, English translation of abstract only.	1-3, 5-8, 13-26
Y	GUYTON. Human Physiology and Mechanisms of Disease, 4th Ed., W.B. Saunders Co. 1987. pages 601-603.	1-3, 5-13
Y	KIRSCHMANN. Nutrition Almanac, 2nd Ed., McGraw-Hill Book Co. 1984. pages 121-123 and 201-202.	1-8, 13, 18, 22
Y	JP 6233669 A (YG et al) 23 August 1994, English translation of abstract only.	27



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/17574

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, EMBASE, BIOSIS, WPIDS, CAPLUS, NAPALERT, GPI WEB CLIENT  
search terms: red yeast, Monascus, monacolin, hypotension, hypertension, fatty liver, thrombosis, stroke,  
hyperlipoproteinemia, cardiovascular, cardiac, xuezhikang, diabetes, obesity, triglyceride, cholesterol, atherosclerosis,  
arteriosclerosis, fermentation, mold extract, beni-koji, cerebral, hypertiglycerid?, rice wine, red mold rice, pen chaw  
kang mu, ben-cao-gang-mu, hong-qu, huo-xue-hua-yu